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## HIGH FREQUENCY MUSCLES USED IN SOUND PRODUCTION BY A KATYDID. I. ORGANIZATION OF THE MOTOR SYSTEM<sup>1</sup>

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One of the most conspicuous insect songs heard in the Eastern United States is that of the katydid, *Neoconocephalus robustus*. *N. robustus*, like other tettigoniids, produces sound by rubbing a scraper on the edge of the right forewing across a set of teeth or file on the underside of the left forewing. The songs of most tettigoniids, and of gryllids which have a similar sound producing mechanism, consist of a series of readily discernible sound pulses, each pulse corresponding to a single stroke of the wings across one another. In a few tettigoniids, including *N. robustus*, the song is unusual in that it is a loud, continuous buzz. Oscillographic analysis of the song of *N. robustus* indicates that it too is composed of a series of discrete sound pulses, but the pulse frequency is sufficiently high, 150–200 per second, that the sound appears continuous to a human listener (Pierce, 1948; Alexander, 1956; Fig. 1 of this paper). This high pulse frequency indicates either that several sound pulses are produced for each wing cycle or that the frequency of wing movements is extraordinarily high. Evidence will be presented to show that the latter is the case; the wing frequency in a singing *N. robustus* is 150–200 per second with one sound pulse being produced per wing cycle.

Wing movements at frequencies exceeding 100 per second have been recorded during flight for a number of insects in several orders (Sotavalta, 1947). Such frequencies have hitherto generally been associated with asynchronous muscle (=myogenic muscle), a type of muscle peculiar to insects in which there is not a direct relation between the frequency of muscle contractions and the frequency of muscle action potentials. In the more usual synchronous muscle (=neurogenic muscle) each contraction is accompanied by one muscle action potential or a burst of action potentials. Generally the frequency of muscle action potentials in asyn-

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chronous muscle is considerably lower than that of the muscle contractions. Apparently the membrane activity of the muscle, indicated by the muscle action potentials, keeps the muscle in an active state during which it can oscillate at a frequency determined principally by the nature of the load (Boettiger, 1960; Pringle, 1967).

Asynchronous muscle has not been found in Orthoptera, the insect order which includes the tettigoniids. It was therefore of considerable interest to determine if the rapidly-contracting muscles used by *N. robustus* to move its wings during singing were synchronous or asynchronous. If the muscles were asynchronous, it would mean that this mechanism of muscle control is more widespread among insects than has been thought. If the muscles proved to be synchronous, their repetition frequency during singing would make them among the fastest synchronous muscles in the animal kingdom.

#### MATERIALS AND METHODS

The animals used were adult male specimens of *Neoconocephalus robustus* collected from salt marshes in Falmouth, Massachusetts. The captured animals were fed lettuce and kept in cages outside the laboratory or near a window so they experienced approximately normal diurnal fluctuations in light intensity.

Muscle action potentials were recorded between electrodes implanted in the thoracic muscles and a bare silver wire in the abdomen. The muscle electrodes were 50  $\mu$  silver wires, insulated except at the tip. They were inserted through holes in the exoskeleton and sealed in place with dental wax. All electrodes were soldered to long leads of copper wire, 80  $\mu$  in diameter. These were light enough that they did not seriously hinder the movements of the animal but strong enough that they usually were not broken by the animal's movements.

Electrode implantation was done under CO<sub>2</sub> anesthetization. After the electrodes were in place, the animals were kept in inverted funnels, 15 cm in diameter, with the recording leads emerging from the spout of the funnel. The animals usually did not sing in the evening following electrode implantation, but they generally did on subsequent evenings. The muscle action potentials were amplified with conventional capacitor-coupled amplifiers and recorded on magnetic tape for later analysis and photography. The position of the recording electrodes was verified by postmortem dissection. In later experiments an electrode marking technique was used to avoid possible misinterpretations caused by electrode movement during the dissection. After an animal had sung and action potentials had been recorded, the animal was anesthetized with CO<sub>2</sub> and current was passed between each of the recording electrodes and the indifferent electrode, the indifferent electrode being the cathode. The animal was then fixed in a solution made of equal parts of 10% formaldehyde and commercial photographic developer (Kodak D19). The developer reduced the silver deposited from the electrodes by the current and left a small black spot to mark the position of the tip of each electrode. A current of 100  $\mu$ A for 10 seconds produced spots easily seen with a dissecting microscope.

The temporal relations between muscle action potentials and wing movements were determined with a strobe light arranged so that it was triggered after a variable delay by the action potentials recorded from one muscle. By adjusting the delay while watching the animal, the strobe light flash could be made to occur when the wings reached the maximally open or maximally closed positions. An electrical

pulse coincident with the light flash was recorded on the magnetic tape along with the muscle action potentials, thus marking the wing position.

## RESULTS

### *The frequency of sound pulses and wing movements*

The song of *N. robustus* typically consists of a series of sound pulses at 150–200 per second. Each pulse is an envelope of sound, the frequency within the envelope being approximately 7 KHz (Fig. 1; Pierce, 1948). It is not known whether the 7 KHz frequency represents a resonant frequency of some part of the sound producing mechanism or the rate at which individual teeth on the file of the left forewing are struck by the scraper on the right wing (for discussion see Dumortier, 1964).

In some tettigoniids two sound pulses are produced for each wing cycle; one when the wings cross one another in closing and the other on the opening stroke (Dumortier, 1964). Alexander (1956) has suggested that the sound pulses of *N. robustus* may be paired with two sound pulses being produced for each wing cycle. If this were the case, the frequency of wing movements should be half that of the sound pulses, that is, 75–100 per second. We examined singing *N. robustus* with a stroboscope and found that in 48 bursts of singing from 10 animals the average wing frequency was 177 cycles per second (range = 145–200 cycles per second, 20–23° C). The correspondence between wing frequency and that of sound pulses indicates that generally one sound pulse is produced on each cycle of wing movement.

Most of the songs which we recorded were from insects in the inverted funnels used to hold animals during measurement of muscle action potentials. These songs were often more complex than those of Figure 1 with two or more sound pulses for each wing cycle. This may be a normal feature of the song, but could also be a result of echoes in the small chamber holding the animals. In some recorded songs occasional sound pulses are reduced or missing (Fig. 2). This suggests that sometimes the wings may move by one another without the scraper encountering the file and so without a sound pulse being produced.

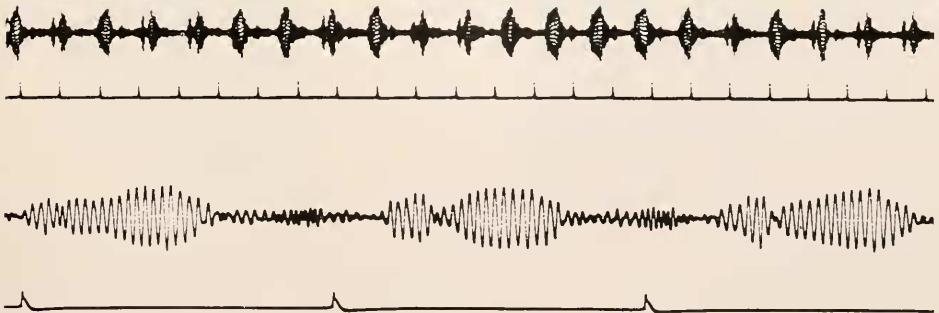


FIGURE 1. Sound pulses produced by a stridulating *N. robustus*. The time marks in this and following figures are 5 msec apart.

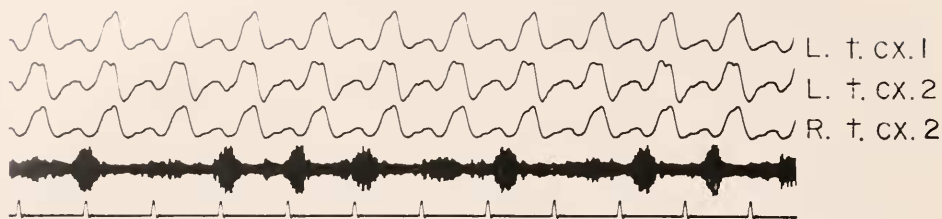


FIGURE 2. Sound pulses and muscle action potentials from three synergistic muscles. In this and other records showing action potentials, positive is up. Note the sound pulses are reduced or absent on some action potential cycles.

### *The frequency of muscle action potentials*

Muscle action potentials recorded with implanted electrodes always occurred in essentially a one-to-one relation to sound pulses and wing movements (Figs. 2, 5, 6). Thus despite the high frequency of the wing movements the muscles used to move the wings are synchronous muscles.

Because of the high repetition rate the muscle action potentials recorded during singing appear as parts of a continuous wave rather than as distinct spikes. The electrical record is also complicated by cross talk from muscles adjacent to that from which the recording is made. Wing musculature makes up most of the mesothoracic volume and large portions of this musculature fire synchronously during singing. Some electrical pickup between muscles is probably unavoidable given that there is simultaneous activity of large blocks of muscle in a relatively small space. Distinct spikes, recorded in only one of several channels, are seen during the warm-up period preceding singing (Heath and Josephson, 1970) and sometimes at the onset or cessation of singing (see Figs. 5, 11, 12). These presumably represent activity in a single muscle with possible contributions from synchronously firing neighbors.

The extracellular action potentials are up to 10 mv in amplitude. The recorded potentials are principally positive when the electrode tip is firmly within the muscle; the potentials from electrodes lying on the surface of a muscle or between two muscles frequently have large negative components. The action potentials often have notches on the rising phase or multiple peaks. This might be due to electrical pickup from neighboring muscles or, more likely, to activity recorded from two or more functional units active nearly simultaneously within single muscles.

The possibility that the recorded potentials are movement artifacts cannot be rigorously ruled out but similar techniques applied elsewhere with insect muscles have been shown to be adequate for recording electrical activity of muscle (*e.g.*, Wilson, 1961; Bentley and Kutsch, 1966). The regularity and repeatability of the wave shape throughout bursts of singing and in successive bursts make it very unlikely that the recorded electrical events are movement artifacts rather than muscle action potentials. The cycle-to-cycle repeatability of the electrical record also indicates that the singing muscles are equally active on each cycle and rules out the possibility that the high frequency is due to an alternation of muscle blocks such that different groups of muscle elements are active on successive cycles.

*The functional organization of the singing musculature*

The muscles used in sound production, those which move the forewings, are shown in Figure 3. The arrangement of wing muscles in the mesothorax of *N. robustus* is similar to that described by Tiegs (1955) for the tettigoniid *Acridopeza reticulata* with the following exceptions: (1) in *Neoconocephalus* there is but a single basalar and single subalar muscle on each side, in *Acridopeza* there are three basalars and two subalars on each side; (2) in *Acridopeza* the tergotrochanteral muscle is of moderate size while in *Neoconocephalus* it is very small and probably does not play a significant role in sound production.

The pattern of electrical activity from the forewing muscles during stridulation is quite simple. All indirect flight muscles (the tergotocoxals, tergotsternal, pleuro-

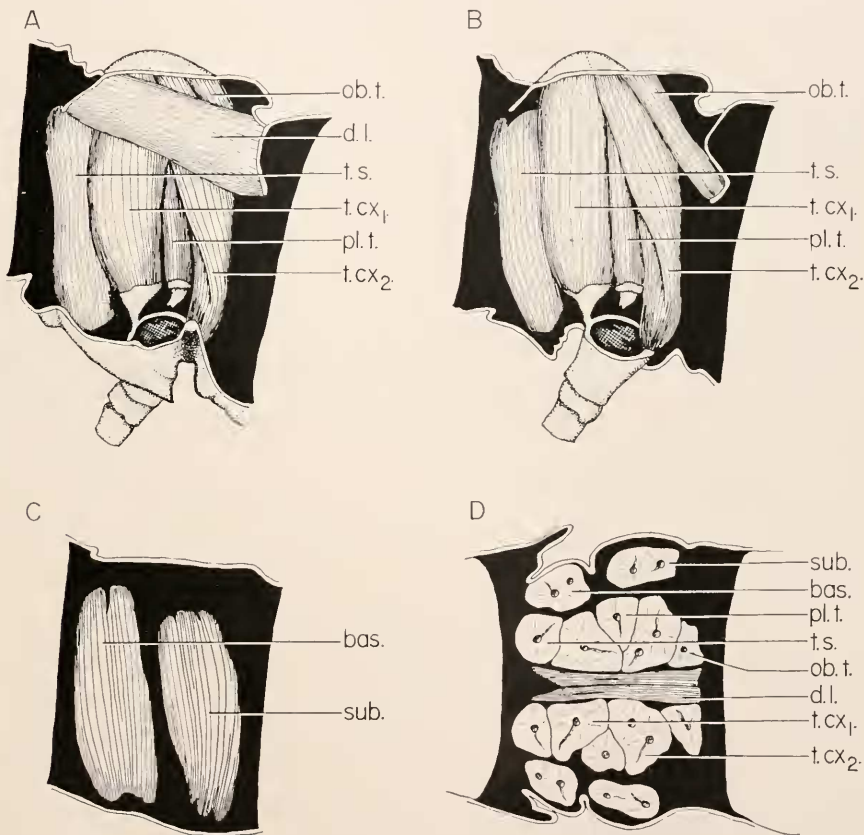


FIGURE 3. The musculature of the mesothorax. A is a median view of the muscles of the right side; anterior is to the left. In B and C progressively more of the medial musculature has been removed to expose lateral muscles. D is a horizontal section through the dorsal mesothorax. The tergotrochanteral muscle is not shown. The two thin branches of this muscle are lateral to the intersection of the first tergotocoxal and the pleurotergal. The abbreviations used are: ob.t., oblique tergal; d.l., dorsal longitudinal; t.s., tergotsternal; t. cx<sub>1</sub>, first tergotocoxal; pl.t., pleurotergal; t. cx<sub>2</sub>, second tergotocoxal; bas., basalar; sub., subalar.

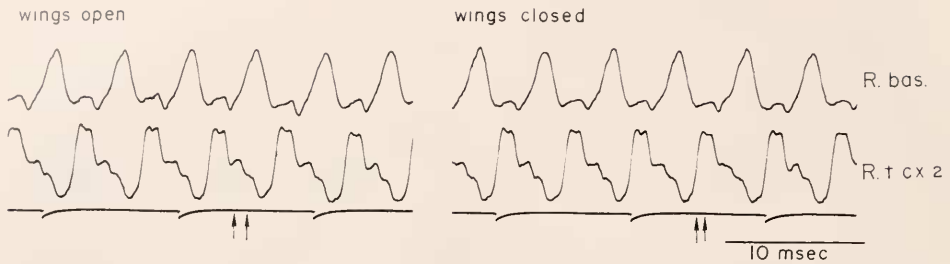


FIGURE 4. Strobe light determinations of wing position during singing. The strobe light was triggered by the muscle action potentials recorded in the upper channel after a delay which was varied so the light flash occurred when the wings were fully opened (left set of records) or fully closed (right set). The lower channel marks the time of the light flash. In these records the strobe light was triggered on every other wing cycle. The arrows mark the range for five separate determinations of wing position with this animal.

tergal, oblique tergal and dorsal longitudinal muscles) fire essentially synchronously. The direct flight muscles (the basalar and subalar muscles) also fire synchronously and in approximate antiphase to the indirect muscles. Stroboscopic determination of wing position during singing indicates that the direct flight muscles begin to fire when the wings are approximately fully opened and the peak of the muscle action potential occurs in the middle of the closing stroke (Fig. 4). Similarly action potentials from the indirect flight muscles begin when the wings are fully closed and the peak occurs during the opening stroke of the wings. Since the next movement following the potential peak from the direct flight muscles is wing opening, the direct flight muscles are presumably wing openers. By the same argument the indirect flight muscles are wing closers. Confirmation of this is given by activity patterns recorded at the cessation of singing. When singing stops, either spontaneously or in response to mechanical disturbance, the wings stop in the closed position. The indirect flight muscles are the last to fire when singing stops (Fig. 5); these must, therefore, be wing closers. When singing resumes, the first wing movement is opening and the first electrical activity appears in the direct flight muscles. These then are wing openers.

In the animal of Figure 4 the latency between the muscle action potential peak and the wing movement initiated by the action potential is approximately 2 msec. In isolated locust flight muscle the latency between the peak of the muscle action potential and the onset of contraction is about 1 msec. and is relatively independent of temperature (Neville and Weis-Fogh, 1963). The longer delay between the action potential and the onset of the initiated movement in *N. robustus* presumably represents time taken for the tension to rise in newly activated muscle until its effect is greater than that of the antagonistic muscles in which tension must be simultaneously falling. Sound pulses generally end about the time of the action potential peak in closer muscles, that is, about the time of maximum wing closure. This indicates that the sound pulses are produced on the closing stroke of the wing cycle.

The functional organization of the stridulation muscles in *N. robustus* is generally similar to that described for crickets (Bentley and Kutsch, 1966; Kutsch,

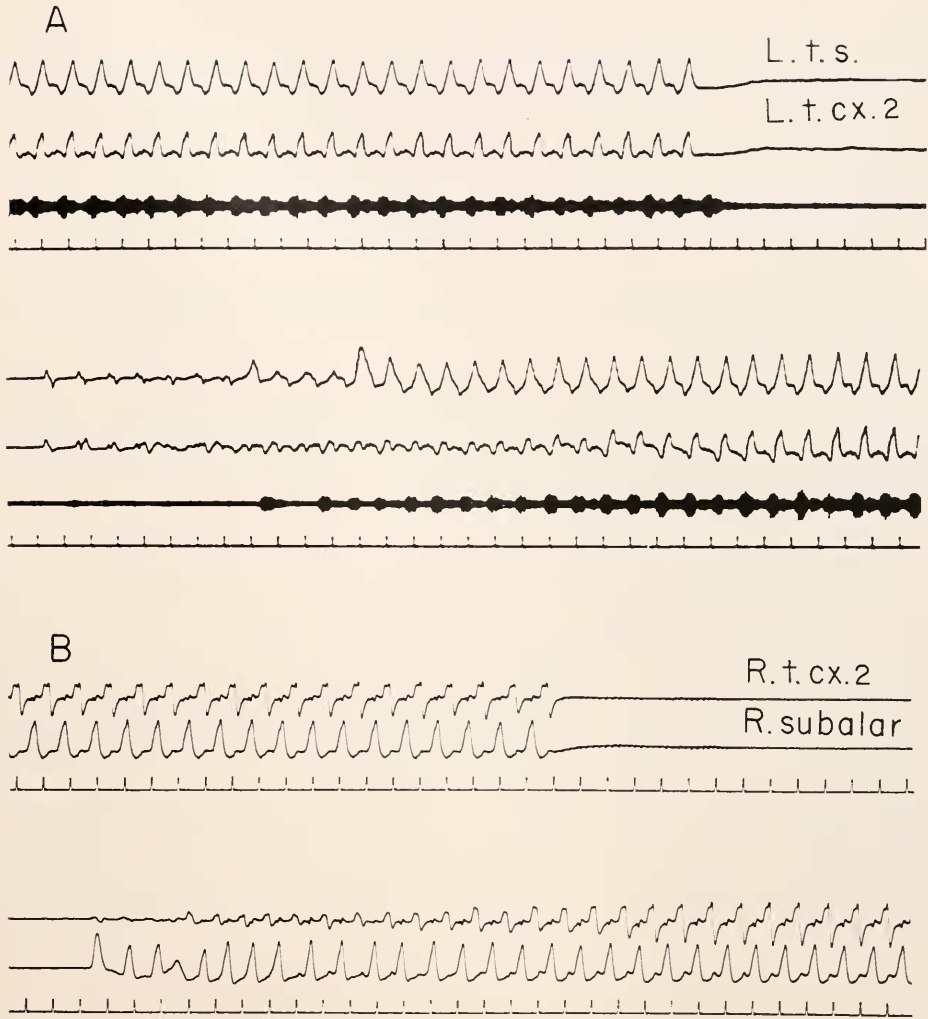


FIGURE 5. Singing pauses induced by tapping on the animal's container. In A and B, the upper sets of records are the cessation and the lower sets the resumption of singing. The singing pause in each case was less than one second.

1969) with direct flight muscles being wing openers, indirect flight muscles principally wing closers, and the sound pulse being produced on the closing stroke of the wings. It is surprising that in *N. robustus* the dorsal-longitudinal muscle is a synergist to the dorsoventral indirect flight muscles, but this is quite clear from the muscle recordings (Fig. 6). The wing movements during stridulation in crickets and tettigoniids are obviously related to those during flight (Bentley and Kutsch, 1966; Huber, 1962), and in insect flight the longitudinal and dorsoventral indirect flight muscles are generally antagonists (Pringle, 1957). There is a precedent for the arrangement found in *N. robustus*. In crickets the dorsal-longi-

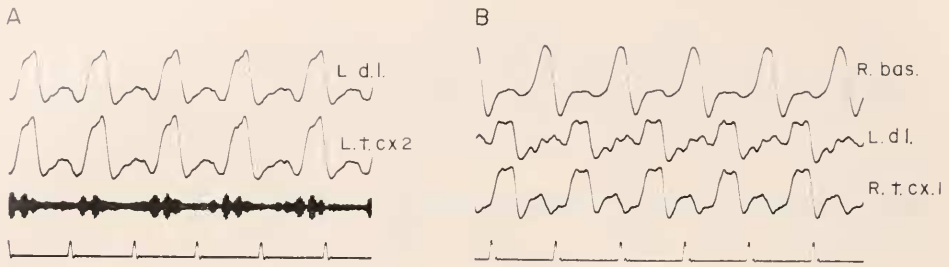


FIGURE 6. Action potentials from the dorsal longitudinal muscle during singing. Note that the dorsal longitudinal clearly fired synchronously with the tergocoxal muscles and is out of phase to the basalar.

tudinal muscle has two components, one of which contracts with the direct flight muscles and the other, as is the case for the single dorsal-longitudinal muscle of *Neocoenocephalus*, contracts with the dorsal-ventral indirect muscles during stridulation (Bentley and Kutsch, 1966; Kutsch, 1969).

#### *Activity patterns during singing*

At the onset of singing muscle action potential patterns can be somewhat erratic. Once singing is fully established, the activity pattern is usually extremely regular. If an oscilloscope sweep is triggered by action potentials from one channel, with most animals successive sweeps fall directly on top of one another, indicating that the action potential frequency is regular and that the shape of the spikes constant from cycle to cycle (Fig. 7A). An electronic counter was used to measure the spike frequency in records collected from 32 animals. Ten intervals, each one second long, were measured for each animal, the intervals being separated by one second periods during which spikes were not counted. The average spike-frequency in these animals was 186.8 per second (s.d. = 13.3 per second, range = 157.6–212.6 per second). The coefficient of variation was computed for each of the animals as a measure of intra-animal variability. The average coefficient of variation was

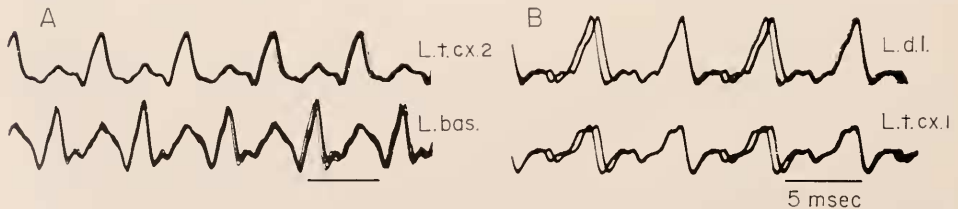


FIGURE 7. Regularity (A) and alternation of cycle lengths (B) during singing. In each case the oscilloscope was triggered by action potentials in the upper channel and the camera shutter left open long enough to superimpose 8–10 successive sweeps. The usual result when this is done is that in A, nearly exact cycle to cycle repeatability. B is from part of a record in which long intervals alternated with short intervals. In this record the oscilloscope sweep was arranged so that one sweep began with a long interval and the next with a short interval. Note that the time taken for any two consecutive cycles was nearly constant as shown by the nearly exact superposition of the second and fourth complete action potentials.



only 0.54% (s.d. = 0.30%). It should be pointed out that this is an underestimate of the regularity. The frequency was determined by counting the number of events in a fixed time interval. This method introduces some variability, for even if the frequency were perfectly constant the number of events counted could vary by one count depending on whether the interval began just before or just after an event. Further, some of the records were from near the onset of singing, a period when the frequency is not stationary but rising slowly (*e.g.*, Fig. 1 of Heath and Josephson, 1970) so the variability here would be greater than that which would be measured when a steady state is reached.

Although the overall frequency is extremely constant, there is sometimes interesting micro-structure in the patterns. In about one-third of the available records the interval between successive action potentials was not constant but alternated between long and short values during some part of the singing period. Alternation, when it occurs, is most pronounced early in the singing period and gradually disappears as singing progresses. Activity in synergistic muscles was always coincident; if there was alternation in the interspike intervals it was the same in each recording channel (Fig. 7B). Alternation in interval length was seen five times in recordings which were made simultaneously from antagonistic muscles. In one of these alternation was seen only in the opener muscle and the closer muscle intervals remained constant. The converse was seen in one animal, with alternation

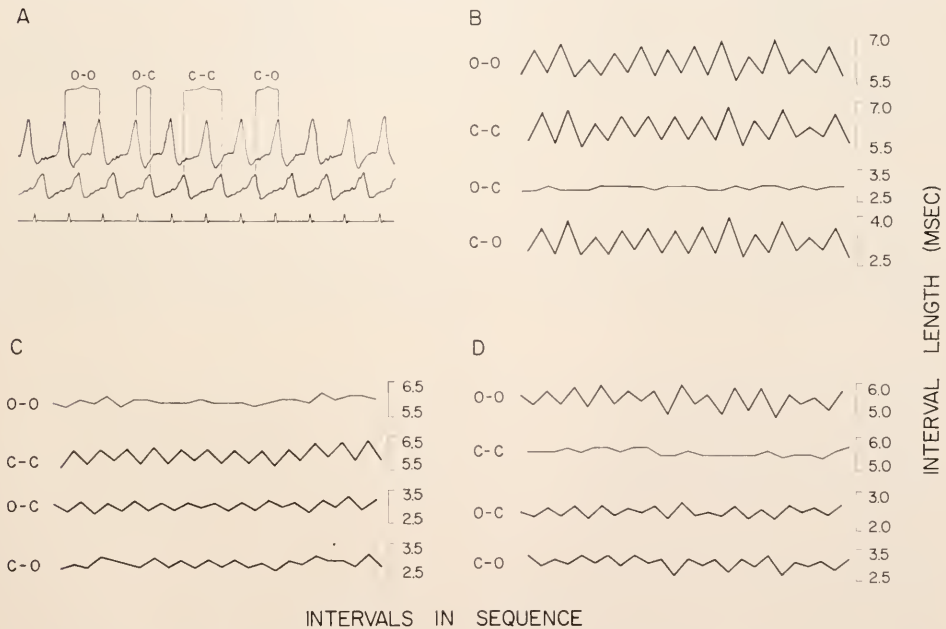


FIGURE 8. Patterns of activity in animals with alternating long and short cycles. The intervals measured are illustrated in A, which shows muscle action potentials from an opener (left subalar, upper channel) and a closer (right second tergocoxal, lower channel). The most extreme alternation encountered is that shown in B. Note that the opener-closer intervals are nearly constant in B as are the opener-opener and closer-closer intervals in C and D, respectively.

being confined to the closer muscle. In three animals alternation occurred in both opener and closer muscles. In these records the intervals between closer action potentials and those between opener action potentials alternated between long and short values but the intervals between an opener spike and the following closer spike were constant. Examples of these patterns are seen in Figure 8 and their implications as to the central organization of elements producing the activity are considered in the discussion.

One animal produced an unusual activity pattern in which rhythmic electrical potentials, obviously in the singing pattern, occurred in only one of two antagonistic forewing muscles being monitored (Fig. 12C). This "singing" occurred in short bursts following apparently normal warm-up activity. No sound was produced. The potentials from the closer muscle were typical of singing. The opener muscle produced no large spikes although these were present during warm-up. Low level activity in the opener channel, however, suggests that other opener muscles were firing in their normal sequence. This indicates that in the command chain there are points of lability at the level of individual muscles or motoneurons which can result in a muscle failing to participate in the usual activity pattern.

#### *Warm-up and the transition to song*

Singing is preceded by a warm-up period during which the forewing muscles are active. During warm-up the wings are held in the resting rather than singing position and normally antagonistic muscles fire synchronously so no wing movement or sound is produced (Fig. 9; Heath and Josephson, 1970). The thoracic temperature rises at about  $1.5^{\circ}\text{C}/\text{min}$  during warm-up and at the onset of singing the thoracic temperature is about  $33.5^{\circ}\text{C}$ .

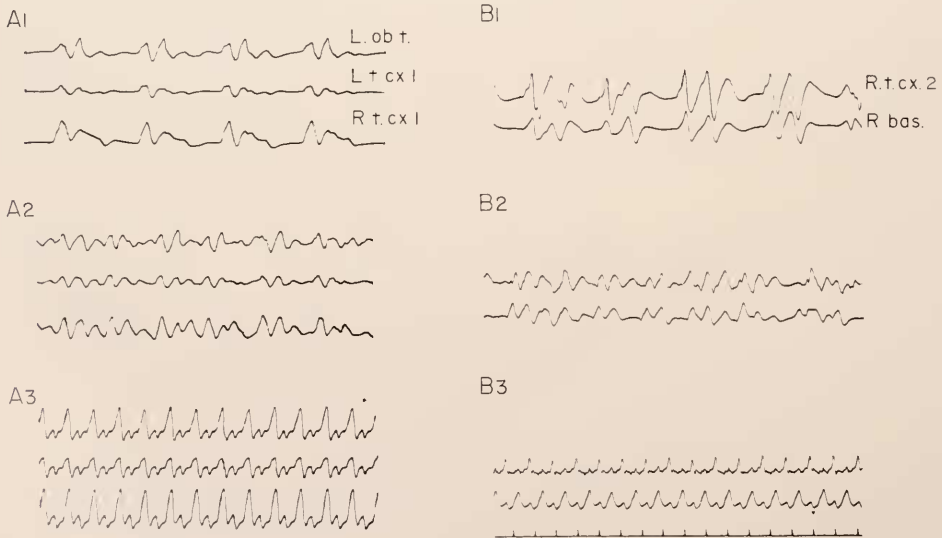


FIGURE 9. Warm-up and singing in synergistic muscles (A) and antagonists (B). The upper sets of records are from early warm-up, the middle sets from late warm-up and the lower sets show fully-established singing. The 5 msec time signal in (B3) applies to all records.

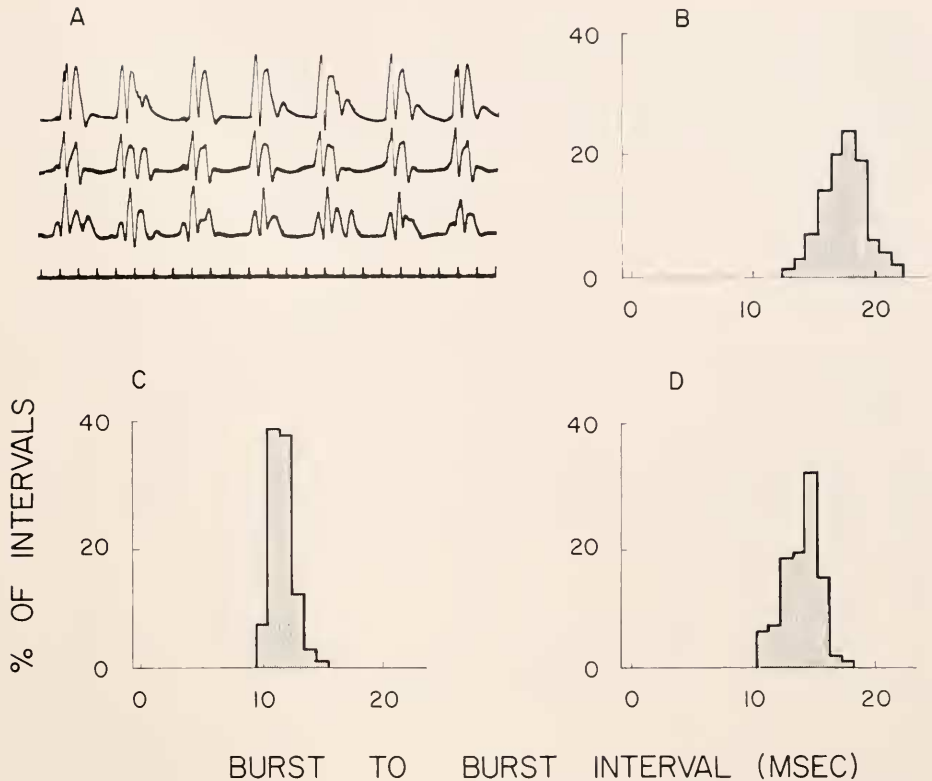


FIGURE 10. Inter-burst interval distribution during early warm-up. Each histogram includes 100 successive intervals measured from the onset of one burst to the onset of the following burst. The records chosen for analysis were ones in which the bursts appeared rather regular in a preliminary inspection. A portion of the original record from which D was obtained is shown in A. The muscles here are the right tergosternal (upper), the right first tergocoxal (middle) and the left dorsal longitudinal (lower channel).

Initially warm-up is intermittent with periods of muscle activity several seconds to a few minutes long separated by silent periods. Later the activity is not interrupted by significant pauses. Through most of warm-up the muscle action potentials occur in short bursts of two to four individual potentials, seen clearly in some records but tending to fuse in others. The interval between the onset of successive bursts is 10–30 msec. The bursts can occur quite regularly (Fig. 10). The bursts might be due to either individual motor units in each muscle firing in slight asynchrony or to multiple firing by the same population of units. The latter seems more likely. The intervals between successive peaks of a burst can be quite regular, suggesting repetitive firing. Further, simultaneous recordings from different muscles usually show exactly the same number and spacing of pulses in equivalent bursts, even when the recordings are made from muscles which are antagonists in singing. This similarity would require that each muscle has the same number of motor units which are activated in the same pattern if the separate peaks are due

to multiple units rather than repetitive firing. As warm-up proceeds, the burst frequency may increase somewhat, but the frequency change is less than might be expected from the rising thoracic temperature. In one animal from which electrical recordings were made the thoracic temperature was also measured with an implanted thermistor (see Heath and Josephson, 1970, for details). In this animal the burst frequency was 56 per second early in warm-up when the thoracic temperature was 26° C and had increased to only 68 per second when the thorax had warmed to 31° C. Muscle contraction frequency during warm-up by the moth, *Hyalophora cecropia*, is similarly only slightly dependent on thoracic temperature (Hanegan and Heath, 1970). Late in warm-up the muscle activity usually loses its bursty character and the recorded potentials appear as a continuous ripple. Regular bursts are interspersed with periods of continuous activity at the transition between bursting and continuous activity. It seems as though the transition from bursting to continuous activity results from an increase in the number of pulses in each burst so that activity continues through the interburst period. One animal produced only short bursts of potentials throughout warm-up; in all others the activity became continuous for some time prior to the onset of singing. Muscle potentials stop abruptly for a brief period just preceding the onset of song (Fig.

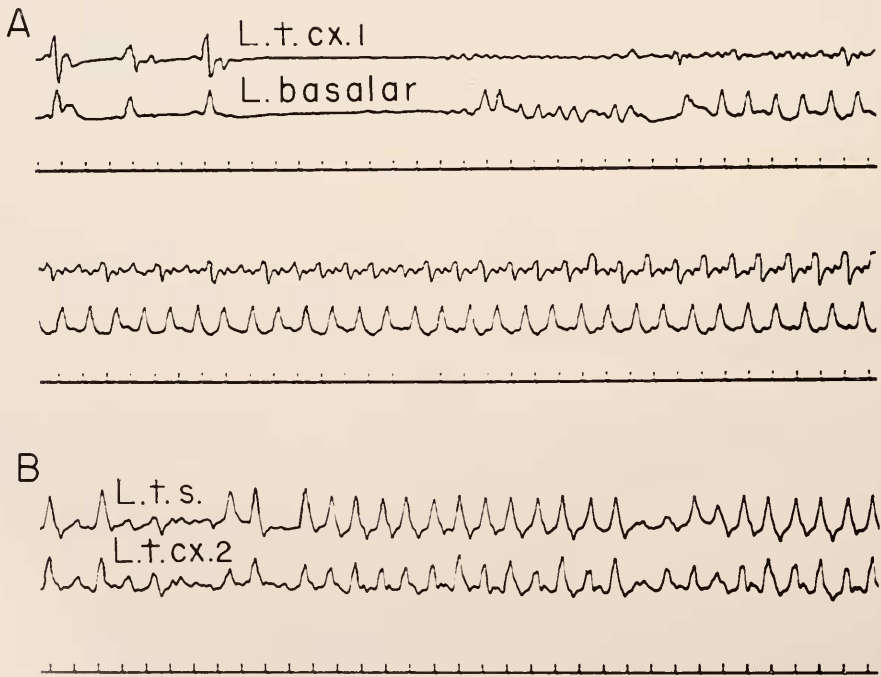


FIGURE 11. The transition from warm-up to song. The lower set of records in A is a continuation of the upper set (part of one cycle is missing). Note the pause in A between the end of warm-up and the onset of singing. Activity recorded near the onset of singing from another animal is shown in B. Here there was missing of occasional cycles. Another example of missing during early singing is seen in Figure 2 of Heath and Josephson (1970).

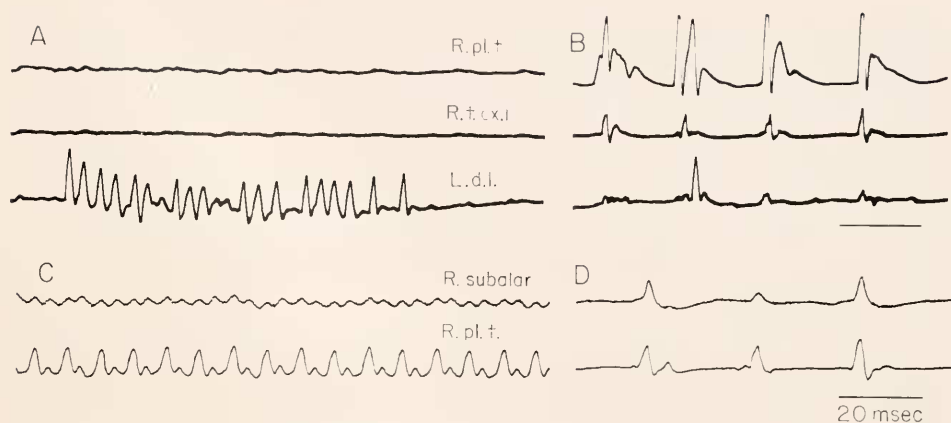


FIGURE 12. Some unusual activity patterns. A is from early warm-up and shows a short burst of spikes restricted to one channel. B was recorded slightly later from the same preparation. It is included because it shows that all three channels were operative and is another example of activity not synchronous in all channels during warm-up. The activity in the lower channel of C was clearly the singing pattern but in the upper channel only low level activity was recorded, indicating that this muscle was not firing. This pattern, which occurred without sound, followed apparently normal warm-up several times in this animal. D is from the same animal shortly later and again shows that both channels were operative.

11A). The duration of the pre-singing pause averaged 64 msec (range = 25–110 msec) in five recorded transitions from warm-up to song.

A striking feature of warm-up is the usual synchrony in activity recorded from different muscles. In all animals occasional bursts may have more or fewer peaks in one channel than in others but, as indicated above, the synchrony usually extends even to the number and spacing of pulses in bursts. It must be emphasized that the deviations from coincident activity now to be considered are exceptional. In two animals, in both of which activity was monitored simultaneously from three muscles, one muscle consistently produced single spikes each time the other two produced bursts. In some animals one muscle produced single spikes and short bursts of spikes, occasionally at frequencies exceeding those of singing, which were not seen in other channels (Fig. 12). While probably not of great significance for singing behavior, these instances of activity isolated to one channel indicate that there is not inescapable coupling between motor neurons during warm-up. Further, recorded events restricted to one channel show that there is often little inter-channel coupling due to electrical fields created within the thorax.

The first rhythmic potentials characteristic of singing are seen in opener muscles but not all opener muscles begin simultaneously; one may fire several times before another begins. Asynchrony in the onset of the singing pattern is even more pronounced in the closer muscles and most forewing muscles may be firing regularly and sound production have begun before an individual closer muscle joins in (Fig. 5A). There may be facilitation of pulse amplitude over a number of cycles after the onset of the singing pattern. This facilitation is more obvious in closer than in opener muscles. In a related tettigoniid, muscle action potentials from isolated forewing muscles show no facilitation (Josephson, in preparation), suggesting that

the increase in action potential amplitude recorded at the onset of singing may result from recruitment of motor units within the muscle, possibly with cross-talk contributions from neighboring muscles. Near the onset of singing, activity recorded from a muscle may occasionally miss a cycle or a number of consecutive cycles, up to 10 (Fig. 11B). This skipping of cycles seems to involve the whole set of synergistic muscles. At the expected time of firing there are no small deflections in the records of the sort which result from cross talk from nearby active muscles. When two synergists are monitored, both skip cycles together while antagonists continue regular firing. This skipping must, therefore, result from lability in some part of the command chain common to the motor neurons forming the synergistic group.

As indicated above, singing usually stops abruptly with the closer muscles being the last to fire. Occasionally the closer muscles produce an extra spike or two at the end of singing and in one animal a single closer muscle continued to fire irregularly after the singing activity had stopped in other channels.

## DISCUSSION

### *Repetition frequencies of synchronous muscles*

Despite the high wing frequency during singing there can be no doubt that the wing muscles of *N. robustus* are synchronous. There is clearly a one-to-one relation between muscle action potentials and wing strokes. Further, the muscle ultrastructure is entirely consistent with that predicted for a fast, synchronous muscle (Elder, 1971). The sarcoplasmic reticulum is extremely well developed and the myofibrils are thin, minimizing diffusion distances from the center of a myofibril to the nearest sarcoplasmic reticulum. Finding a muscle action potential for each contraction does not necessarily mean that there is an antecedent motoneurone impulse for each contraction. In bumble bee flight muscle a single motoneurone impulse can evoke a junctional potential which produces several muscle action potentials (Ikeda and Boettiger, 1965). The strict synchrony between synergistic muscles in *N. robustus*, however, cannot be accounted for on the basis of repetitive firing in independent units; some central synchronizing mechanism is necessary. Thus in *N. robustus* there is almost certainly a one-to-one relation between contractions and impulses in participating motoneurones; the muscles are, as is the usual case, both synchronous and neurogenic.

The wing frequency of *N. robustus* during singing is 145–212 per second. In contrast, the highest wing frequency during flight reported for animals presumably using synchronous muscles is that of some moths (Aegeridae) whose wing frequency reaches or slightly exceeds 100 per second (Sotavalta, 1947). It is interesting that with the exception of these moths and the asynchronous muscles of insects, all muscles which have been found to have repetition frequencies exceeding 100 per second are involved in sound production. The synchronous tymbal muscles of cicadas (Hagiwara, 1956; Aidley, 1969) and the sound producing muscles of the toadfish, *Opsanus tau*, (Skogland, 1961), the squirrel fish, *Holocentrus rufus*, (Gainer, Kusano and Mathewson, 1965; Winn and Marshall, 1963), and the lobster, *Homarus americanus*, (Fish, 1966; Mendelson, 1969) all contract at frequencies near to or greater than 100 per second during sound production. The sound producing muscles of midshipman, *Porichthys notatus*, (Cohen and Winn, 1967) and

the cricothyroid muscle of the bats *Epitesicus fuscus* and *Myotis lucifigus* (Revel, 1962; Griffin, 1958) can reach or exceed contraction frequencies of 200 per second. The performance of sound-producing muscles in fish and in the lobster is the more remarkable when it is considered that the high frequencies are achieved at temperatures considerably lower than is the case with bats or with *N. robustus* in which the thoracic temperature is about 35° C during singing (Heath and Josephson, 1970). Indeed the sound producing muscle of the lobster has the most extensively developed sarcoplasmic reticulum yet described; here the sarcoplasmic reticulum comprises approximately 75% of the muscle volume (Rosenbluth, 1969). However, it is an insect which seems to offer the highest repetition frequency yet found for synchronous muscle. The bush cricket, *Orocharis gryllodes*, stridulates with its forewings. The wing frequency during stridulation is a function of ambient temperature and reaches 280 per second at 35° C (Walker, 1969). Although it has not been directly demonstrated it seems likely that the singing muscle is synchronous because (1) asynchronous muscle has not been found in the large number of orthopteran insects which have been examined, and (2) of our results in this paper indicating that insect synchronous muscle can exceed repetition frequencies of 200 per second.

#### *The endogenous origin of the motor patterns*

The forewing muscles in the mesothorax of *N. robustus* are involved in several activity patterns which differ in the frequency of muscle activation, the temporal organization of the muscle action potentials (single impulses at regular frequency or impulse bursts) and the phase relations between different muscles. Two activity patterns, those of warm-up and singing, are described here. The same muscles involved in these are also used for flight, during which the muscles are driven at a much lower frequency than during singing and in different phase relations than during warm-up. The tergo-coxal muscles, from their morphology, may also be used during walking; the first tergo-coxals being coxal protractors and the second tergo-coxals being coxal retractors (Fig. 3). If these muscles are used during walking they may simultaneously participate in two activity patterns for *N. robustus* frequently walks about during warm-up and it can shift its position while singing without there being a noticeable pause in the song.

Three basic mechanisms have been proposed for the origin of rhythmic motor patterns in animals (for reviews see Wilson, 1964; Hoyle, 1964): (1) Reflex chains in which the consequences of the motor activity in one part of the cycle initiate sensory inflow which triggers the next part of the cycle. (2) Stored patterns of expected sensory input (sensory tapes (Hoyle, 1964) or sensory templates (Wilson, 1968a)) to which actual sensory input is compared, any discrepancies initiating a compensating motor output. (3) Endogenous generators which produce repetitive series of appropriate motor commands without the necessity of sensory inputs as timing cues. In the last, sensory information does not have a direct role in the sequencing although it may be necessary to initiate a pattern and it may modulate activity in progress. The stored set of commands has been termed a motor tape (Hoyle, 1964) or a motor score (Wilson, 1968a).

Of these possibilities the last, that of endogenous motor pattern generators, has been documented most thoroughly. Particularly pertinent examples here come from studies of insect flight and sound production. In locusts and moths the basic

pattern of commands to wing muscles during flight is not altered by total or partial deafferentation indicating that there is an endogenous program which produces the appropriate command patterns (Wilson, 1961; Kammer, 1967). Fixing the wings, changing the wing loading, or total deafferentation does not essentially change the muscle activity patterns of crickets attempting to sing, again indicating that the motor activity results from an endogenous pattern generator (Kutsch and Huber, 1970; Bentley, 1969b). There are several reasons for believing that the activity patterns of warm-up and song in *N. robustus* also result from endogenous mechanisms which generate motor output patterns. Warm-up occurs with the wings folded in the normal resting position and with no obvious movement of the wings or other thoracic structures. Thus sensory information about wing position is not available for reflex arcs or for comparison with sensory templates. It is possible that receptors measuring muscle tension or deformation of the thoracic exoskeleton could give timing cues but the organization of warm-up activity, with simultaneous contraction of antagonistic muscles, seems designed to minimize thoracic movement and makes it seem unlikely that proprioceptors play a necessary role in the generation of the pattern. The short cycle length during singing poses problems for reflex chain or sensory template models for there is insufficient time during a cycle to initiate and process sensory information affecting later parts of the cycle. This does not completely rule out schemes requiring sensory input, however. For example, it could be proposed that singing results from a peculiar reflex chain in which sensory input indicating wing closure triggers not the next wing opening but the wing opening one or more cycles later. The most compelling evidence for an endogenous pattern generator is the observation that a set of synergistic muscles can skip one or several consecutive cycles without disrupting the rhythm, the next muscle action potential following a skipped cycle appearing just where it would be predicted on the basis of the intervals preceding the skip. Were the pattern generated by a reflex chain, a skipped cycle should terminate the rhythm; were a sensory template involved, a skipped cycle would result in an unusually large discrepancy between actual and expected sensory input and hence an altered motor output. Neither is the case. Thus singing and probably warm-up as well in *N. robustus* are members of a growing list of activities controlled by endogenous generators of motor patterns.

#### *The mechanism generating the singing pattern*

In our speculations about the mechanisms producing the motor output patterns of singing we will assume that information flows along a sequential set of elements. An element may be a single neuron or a group of neurones which modifies the information in a particular way. We will also assume that feedback from distal to proximal points in the command chains does not play an essential role. Because of the short cycle length there is little time for processing feedback information and, pragmatically, a seemingly satisfactory model can be proposed that does not require feedback. Particular attention will be given to irregularities sometimes seen in the singing pattern, specifically the alternation between long and short intervals. The mechanism generating this alternation is not known. Intuitively it seems possible that a neuron receiving a regular, phasic input could respond with pulses in alternating intervals if: (1) the intervals between inputs were close to the neurone's



refractory period, and (2) there was post-firing depression in the neurone, the effects of which lasted more than a single cycle and the magnitude of which was greater the shorter the interval between firings. Thus if an interval happened to be shorter than usual, the excitability after firing would be lower and the next interval longer than average. Conversely after a long interval there would be less depression so the next interval would be short. Of course one can offer schemes in which a multicellular network responds with alternating intervals. But whatever way such patterns are produced, they are useful as markers, for when introduced into the command chain they will be propagated along the chain unless they reach some portion which smoothes out irregularities. As an example of the use of alternation as a marker consider the pattern shown in Figure 8C. Here the alternation is confined to the closer channel. This indicates that there is a point of lability at which alternation can originate distal to portions of the command chain shared by the opener and closer channels.

The following seem likely components of the central mechanism generating the activity patterns of singing. The scheme proposed is summarized in Figure 13. (1) Each forewing muscle is innervated by a set of motoneurons, some or all of which are involved in singing. Judging by the innervation patterns of other insects each motoneurone probably innervates only one muscle (*e.g.*, Bentley, 1970). This is partially confirmed in *N. robustus* by the unit activity sometimes recorded

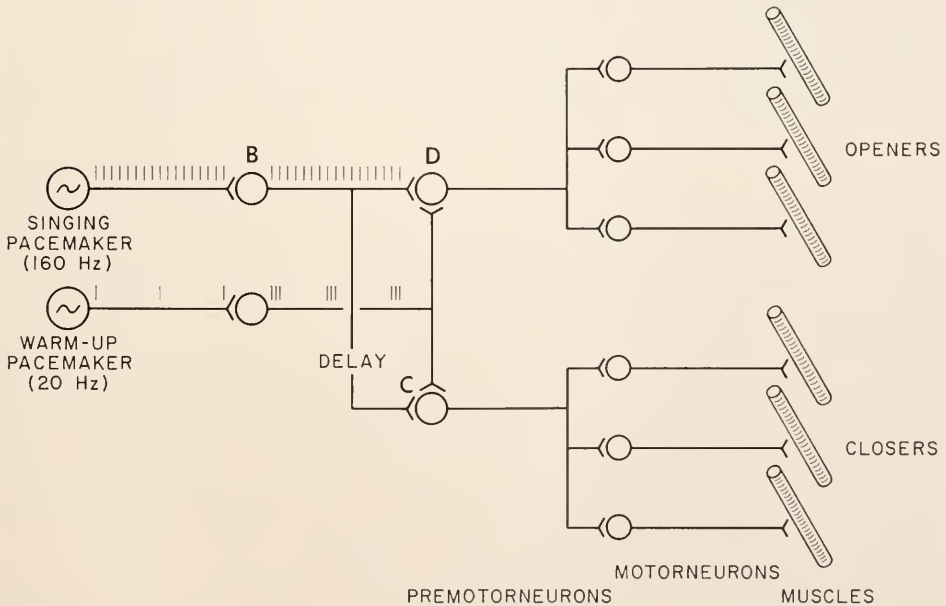


FIGURE 13. The model proposed for the generation of singing and warm-up motor patterns. The short vertical bars to the left are the spike patterns expected from those parts of the circuit. The singing and warm-up pacemakers may be mutually inhibitory since they do not simultaneously contribute to the output. B, C and D indicate the points in the circuit at which alternation might originate to create the interval patterns shown in Figures 8B, C and D, respectively.

in only one of several channels. (2) There is probably an interneurone or a group of tightly coupled interneurons which can simultaneously activate those opener motoneurons involved in singing and, similarly, an interneurone or group of interneurons which can simultaneously activate the closer motoneurons. For convenience these elements will be called the opener premotoneurone and the closer premotoneurone, even though it is recognized that each may consist of several nerve cells arranged serially or in parallel. The presence of such elements may be inferred from the strict synchrony in action potentials from all synergistic muscles during singing. Ordinary synaptic couplings between synergistic motoneurons, with their attendant delays, would not seem adequate for the coincident firing. Electrical coupling between motoneurons like that which insures synchronous firing among electromotoneurons in a weakly-electric fish (Bennett, Pappas, Aljure, and Nakajima, 1967) is a possible mechanism for simultaneous firing. Electrical coupling between motoneurons has been found in insects (Kendig, 1968; Bentley, 1969a). If there is electrical coupling between motoneurons in *N. robustus* it must not be very tight coupling for sometimes during warm-up and at the onset of singing one motoneurone can fire independently of its synergists. Further, the two tergo-coxal muscles which are synergists during singing are probably antagonists during walking. If singing and walking involve the same motor units these must be capable of independent activity. For these reasons we suggest that the synchrony between synergists is a result of common excitatory input from premotoneurons, possibly augmented by electrical coupling. Interval alternation affecting all muscles in the synergistic group but not the antagonists of the group must begin at the premotoneurone level. (3) The opener and closer premotoneurons are driven from a common source which fires at the singing frequency but there is a fixed delay of approximately 3 msec introduced into the closer portion of the command chain. The presence of a common driver for both opener and closer premotoneurons is indicated by the activity pattern shown in Figure 8b. Similar patterns were recorded from two other animals. In these records both the openers and the closers had alternating long-short intervals and the closer interval lengths were nearly exactly the same as those of the opener intervals in which they began. The result is that irregularities in the opener channel are precisely reflected in the closer channel one-half cycle later. These irregularities must arise in some part of the command pathway common to both opener and closer premotoneurons. The reason for postulating a fixed delay in the closer pathway is obvious from Figure 8B. Although the opener and closer intervals fluctuate considerably the interval between opener and closer muscle action potentials is essentially constant. A fixed delay could be achieved in several ways. It might result from a single additional synapse inserted into the closer chain. Or the input to the opener premotoneurone might simultaneously inhibit the closer premotoneurone, the closer premotoneurone then firing as it escapes from inhibition several milliseconds after the opener premotoneurone has fired. The latter is essentially the explanation offered by Bentley (1969b) to account for the fixed delay between opener and closer muscle firing during cricket stridulation. This delay, like that in *N. robustus*, is of fixed duration although it is considerably longer than that found in the katydid (sixteen as opposed to three milliseconds).

Were it not for the alternating interval pattern recorded from one animal, that

of Figure 8D, the fixed delay between opener and closer activity could be ascribed to transit time across an excitatory synapse from the opener to the closer premotorneurone. In this record there is alternation in the opener but not the closer channel. To interpret this one needs to know if the alternation occurred in all opener muscles, and therefore arose at the premotorneurone level, or if the alternation was restricted to one opener channel and therefore originated at the motorneurone level. Although only one opener muscle was monitored in this preparation it is reasonably certain that the alternation occurred in a number of opener muscles. With the method used some part of the recorded signal results from activity in muscles near the one containing the electrode. The contributions from nearby muscles should appear at different times in the main signal if the muscle being monitored were changing its phase relations to other openers. But the potentials actually recorded had essentially the same shape during each cycle, suggesting that synchrony between openers was maintained and that all openers were responding with similar alternating intervals (see the lower muscle record of Figure 9B3 which was from this animal). Further, the electrical record from the closer muscle of this animal had small spikes between the major closer spikes. The small spikes result from electrical pick up of opener activity. The intervals between the small spikes too alternated between long and short values. Since the small spikes are probably crosstalk resulting from the summed activity of several openers this again indicates that a number of opener muscles took part in the alternation and that alternation originated in the opener premotorneurone. But alternation did not occur in the closer muscle, indicating that the closer premotorneurone is not directly driven by the opener premotorneurone. Thus the portions of the command chain which are common to the opener and closer muscles must precede the premotorneurone level.

#### *Generation of warm-up activity*

Thoracic warming by muscular activity, similar to that seen in *N. robustus* before singing, has been found to precede flight in a number of insects (see Kammer, 1968; McCrea and Heath, 1971; and references therein). The strategy of warm-up appears to be to produce heat by contraction of wing musculature without producing full-scale wing movements. Wing movements before appropriately high thoracic temperatures are reached are likely to be ineffective (the power available from cool muscles may not be sufficient to maintain flight; a low pulse frequency in song may not be seductive to females) and they can attract the attention of predators. Wing movements during warm-up are minimized in several ways. In animals with asynchronous muscles the wings may be folded and mechanically uncoupled from the musculature during warm-up (Leston, Pringle and White, 1965). In animals with synchronous muscles the phase relations between flight antagonists are altered during warm-up. In some moths and butterflies, as in *N. robustus*, all units are activated nearly synchronously during warm-up. In other moths there are phase changes so that some units fire synchronously with normal antagonists and in anti-phase to normal synergists (Kammer, 1968, 1970; Hanegan and Heath, 1970). Kammer (1968) raised the possibility that neuronal circuitry generating motor patterns may be temperature sensitive so that the transition between phase relations characteristic of warm-up to those of flight are an automatic consequence of rising

temperature. In *N. robustus* warm-up activity completely stops and there is a delay of variable duration before singing begins. Similarly there is sometimes a delay between warm-up and the onset of flight in the moth *Hyalophora cecropia* (Hanegan and Heath, 1970). The occurrence of a delay suggests that the transition from warm-up to singing or flight involves more than a temperature-sensitive transition between two output patterns from a single generator. A model in which temperature receptors and higher order integrative centers control the output of separate warm-up and flight pattern generators is proposed by Hanegan and Heath (1970).

The overall synchrony in muscle activity during warm-up suggests that both premotorneurons are then being driven by the same source. One possibility for the warm-up pattern would be an element firing at 10–20 per second which triggers short bursts in a follower which in turn drives both opener and closer premotorneurons (Fig. 13).

In summary, the model proposed for warm-up and song in *N. robustus* contains two central pacemakers, one which provides the singing frequency of 150–200 per second and one which fires at 10–20 per second giving the burst frequency during warm-up. There are probably at least three neuronal elements in series between the forewing muscles and the pacemakers; motorneurons, premotorneurons, and an element which activates the two premotorneurons.

#### *Comparison with some related behaviors*

Mechanisms of sound production in insects have been most extensively investigated in crickets. Singing is considerably more complex in the cricket species which have been studied than in *N. robustus*. Each species produces several distinct songs and in each song the muscle action potential patterns contain a number of frequency components rather than just one as is the case in the only known song of *N. robustus*. For example, in the calling song of crickets, which is functionally equivalent to the song of *N. robustus*, the singing muscles may fire several times per wing stroke and the sound pulses produced by individual wing strokes are grouped in chirps with quiet periods in between (Ewing and Hoyle, 1965; Bentley and Kutsch, 1966; Bentley, 1969b; Kutsch, 1969). On the basis of intracellular recordings from motorneurons and interneurons in singing crickets Bentley (1969b) has proposed that the chirp rhythm is determined by a central oscillator while activity patterns within a chirp result from excitatory interactions between the motorneurons, possibly mediated in part by interneurons driven by the motorneurons. The models proposed for singing in crickets and *N. robustus* differ in detail but they do contain an essential similarity; in each there is assumed to be a central oscillator which operates without feedback from the motorneurons and whose output, through interneurons, triggers the motorneurons.

Muscle activity patterns during insect flight have been extensively studied by D. M. Wilson and his colleagues (for a review see Wilson, 1968b). In the locust, *Schistocerca*, the basic motor pattern is endogenously generated but sensory input can alter the frequency and, through reflexes which are slowly acting with respect to the flight frequency, modify the power output on the two sides to insure flight stability (Wilson, 1961, 1968a). In *Schistocerca* the phase relations between antagonists remain constant over a rather wide frequency range (Waldron, 1967);

this is unlike the singing pattern in *N. robustus* and crickets (Bentley, 1969b) where the time between opener and closer firings is relatively constant and therefore the phase of closer firing changes with changes in the opener-opener interval. To meet the criterion of neuronal economy the flight pattern in locusts has been explained largely on the basis of interactions between neurones at one level, possibly the motoneurones themselves (Wilson, 1966). While the rhythm and phasing between elements of the flight system can be explained on the basis of excitatory and inhibitory interactions between elements at a single level, a hierarchical arrangement of participating neurones, like that proposed here for *N. robustus*, cannot be ruled out. Indeed, as Wilson (1968b) points out, a hierarchical organization is an appealing way to account for the ability of motoneurones to participate with different activity patterns in several behaviors.

#### SUMMARY

1. During stridulation the forewings of *Neoconocephalus robustus* are rubbed against one another at a frequency of 145–212 per second. Despite the high frequency the forewing muscles are synchronous muscles; each contraction is preceded by a muscle action potential.

2. The direct flight muscles of the mesothorax are wing openers during singing; the indirect flight muscles are wing closers. The sound pulse is produced on the closing stroke of the wings.

3. Singing is preceded by warm-up during which all forewing muscles are activated synchronously. In early warm-up the muscles are activated in short bursts, often at a regular frequency. Later warm-up activity is continuous. Muscle activity stops briefly at the transition from warm-up to singing.

4. Muscle activity patterns during singing indicate that the motor output results from an endogenous pacemaker which fires at the singing frequency. There are probably at least three neuronal elements in series between the pacemaker and the forewing muscles. The phasing between opener and closer muscles results from a fixed delay of approximately 3 msec between opener and closer portions of the command chain.

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